

Supplemental Data

Hepatitis C Virus NS5A Protein Down-Regulates the Expression of Spindle Gene *Aspm* Through PKR-p38 Signaling Pathway

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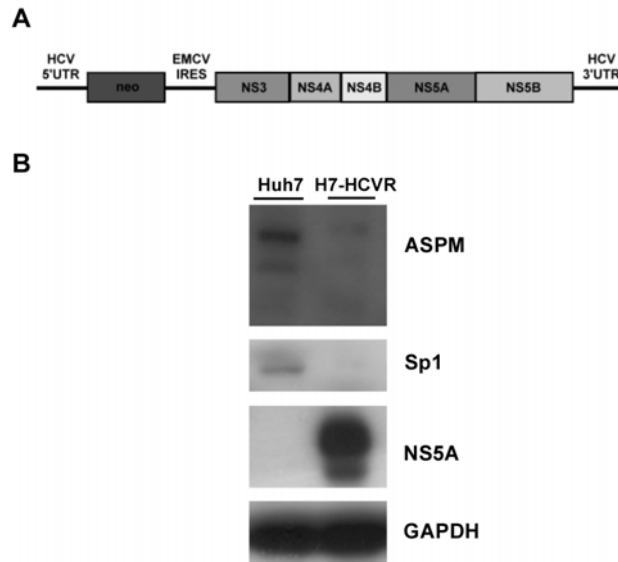


FIGURE S1. Down-regulation of ASPM protein in HCV genotype 1b subgenomic replicon (H7-HCVR cells). A. schematic representation of the HCV type 1b subgenomic replicon construct. The HCV 5'UTR is positioned upstream of the gene encoding neomycin phosphotransferase (neo). The internal ribosome entry site (IRES) of encephalomyocarditis virus (EMCV) directs translation of the HCV polyprotein from NS3 to NS5B region that is flanked at 3' end by HCV 3'UTR. B. expression of the ASPM protein in H7-HCVR cells. Cell lysates prepared from H7-HCVR and parental Huh7 cells were subjected to Western blot analysis with antibodies specific to ASPM, Sp1, NS5A and GAPDH as indicated.

Human	KRSLWDTXXXXXSASTSHNRRVSNIQVVKTFVSQKVDVRVRSPLQACENLAMNEGGPP	206
Mouse	KRSLWNTSKKIP--ASSKHTKRTSKNQHFNESFTISQK-DRIIRSPQPENLAMSECCSSP	195
Human	TENNSLILEENKIPISPIPAFNECHGATCLPLSVRRSTTYSSLHASENRELLNVHSANY	266
Mouse	TEN-----KVPTPSISP-IRECQSETCLPLFLRESTAYSSLHESENTQNLKVQDASI	246
Human	SKV-SFNEKAVTETSFNYSVNVGQRGENSKLSLTPNCSSTLNITQSQIHFLSPDSFVNNS	325
Mouse	SQTFDFNEEVANETFINPISVCHQSEGDRKLTLPNCSSPLNSTQTQIHFLSPDSFVNNR	306
Human	HGANNELELYTCLSSDMFMKDNSQPVHLESTIAHEIYQKILSPDSFIKDNYGLNQDLESE	385
Mouse	YTSNDLKSMMKNVLSDFRKPDAESVCLESQTVHEVCQTILSPDSFLNDNYGLKGLNFK	366
Human	SVNPILSPNQFLKDNMAYMCTSQQTCKVPLSNENSQVPQSPEDWRKSEVSPRIPECQGSK	445
Mouse	SVNPVLSPTQFVKDSMGHV--GQQTGK--SNEASQ-----DWRINEGLAYTPECQHAQ	415
Human	SPKAI FEEL--VEMKSNYYSFIKQNNPKFSAVQDISSHSHNKPKRRRPILSATVTKRKRAT	503
Mouse	TPSSRSEKQNPVEVKPHTYDFTKQK-PKISEFQDAFCHQSKQPKRRRPILSATVTKRKRPT	474

FIGURE S2. Schematic representation of the partial sequences of human and mouse ASPM proteins. A peptide PKRRPILSATVTKRK was synthesized and used as the immunogen to generate an antiserum specific for both human and mouse ASPM. The peptide as indicated is highly antigenic and conserved between human and mouse ASPM proteins.

HCV NS5A Inhibits the Expression of *Aspm* Gene

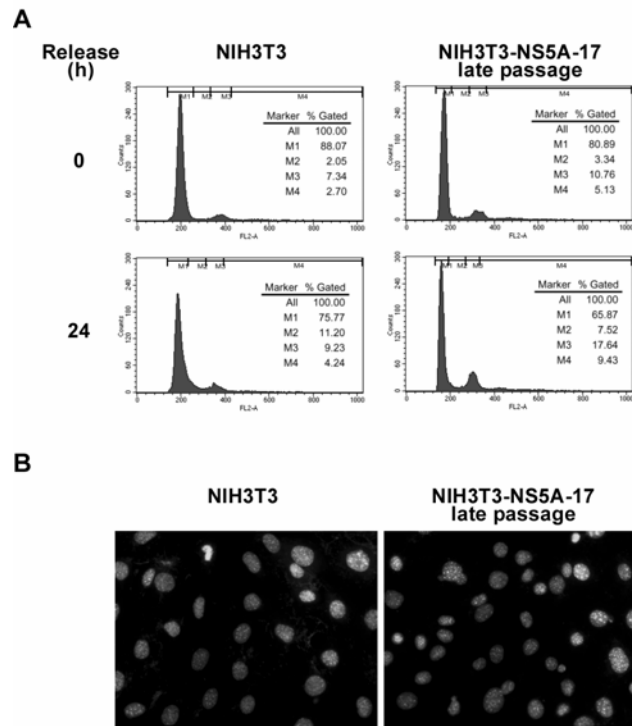


FIGURE S3. The aneuploidy in NS5A-expressing cells. A. detection of multiple nuclei in NS5A-expressing cells by flow cytometry. The late passage NIH3T3-NS5A-17 cells and the parental NIH3T3 cells were arrested at the G₁/S boundary by serum starvation. At 0 and 24 h after release from the serum starvation, 10,000 cells were gated for cell cycle analysis. M1: G₁ phase, M2: S phase, M3: G₂/M phase, M4: multiple nucleus phase. **B.** analysis of mitotic dysregulation in NS5A-expressing cells by immunofluorescence assay. The late passage NIH3T3-NS5A-17 cells and the parental NIH3T3 cells were fixed, permeabilized with 0.5% Triton X-100, and stained with Hoechst dye.